

range. Not only is their geographical distribution often extensive but the vertical range of the various forms exceeds that of the majority of the higher forms of life.

4. While no pathogenic property was noted in any of the forms isolated, other physiological characters, as the formation of soluble enzymes and the ability to reduce nitrate salts, were conspicuous characteristics of the more prevalent forms.

*University of Wisconsin.*

EXPLANATION OF PLATE XXXVI.—Figures of bacteria in cultures drawn with a Zeiss microscope, tube length 160<sup>mm</sup>, no. 6 compensating ocular and  $\frac{1}{13}$  homogeneous oil immersion.

- Fig. 1. *A. Bacillus limicola*, gelatin culture 5 days old.  
 Fig. 1. *B. a*, Short type of cells from agar culture; *b*, lanceolate type from old agar culture; *c*, normal bacillus type from agar culture, 3 days old.  
 Fig. 2. *A. Bacillus pelagicus*, culture in gelatin.  
 Fig. 2. *B.* Spore bearing bacilli from gelatin culture, two weeks old.  
 Fig. 3. *A. Bacillus maritimus*, gelatin culture, 2 days old.  
 Fig. 3. *B.* Single cells from potato culture, 2 days old.  
 Fig. 4. *A. Bacillus litorosus*, 3 days growth in gelatin.  
 Fig. 4. *B.* Cells from young gelatin culture.

## Studies in the biology of the Uredineæ. I.

### Notes on germination.

M. A. CARLETON.

WITH PLATES XXXVII-XXXIX.

Within the last eighteen months I have made about four hundred cultures of uredineal spores, in various media, at various seasons, in order (1) to determine as many facts as possible concerning their biology on the one hand, and on the other hand, by observing the various effects of different chemicals upon their germination, (2) to obtain a working basis for the preparation of fungicides for the prevention of the attacks of certain economic species. A great part of the work, particularly that of most economic importance, has already been reported.<sup>1</sup> What I shall present here, are some results and suggestions of a more technical nature.

Nearly all the cultures made were of the ordinary drop culture style, prepared by depending a drop of the employed medium, in which the spores were immersed, from the lower surface of a cover-glass placed over an ordinary glass cell

<sup>1</sup>Kansas Agric. Exp. Sta. Bull. 38. Mar. 1893.



made by glueing a glass ring to a slide. The bottom of the cell had previously been moistened with water, so that a miniature damp chamber was thus formed. In order to more certainly guard against drying, the further precaution was observed, of placing the cultures themselves in a large damp chamber. Occasionally, simple watch glass cultures were prepared, but these were seldom found to be useful, on account of their great liability to contamination.

#### 1. Effects of different chemicals upon germination.

In carrying on the experiments in this line, one or more check cultures in water were always included in every series of cultures prepared at any one time. The results of the cultures in the various solutions were compared with those of the water cultures, and in every series where germination failed, both in water and in the other solutions, the whole experiment was discarded, of course. The uredospores of grain rusts were used in most of the experiments, particularly the uredo of *Puccinia coronata* Corda, since that species was the most abundant for the longest period in 1892, when the majority of these experiments were performed. In the following tables I give the names of those solutions only which show the most striking results, either favorable or unfavorable. The tables represent only a small proportion of the cultures actually made. The numbers in the column "strength" give the number of parts, by weight, of the commercially prepared chemicals to 10,000 parts of distilled water, in which they were dissolved. The first table shows results obtained with uredospores of *Puccinia Rubigo-vera* (DC.) Wint.:



TIME (hrs.)	MEDIUM.	STRENGTH.	RESULTS.
21	Ferrous sulphate,	10	Not very good.
21	Potassium sulphide,	10	Fairly good.
21	Copper sulphate,	10	Failure.
18	Hydrogen peroxide, (0.3 per cent. sol.),	10	Better than in water.
18	Potassium sulphide,	10	Not very good.
18	Copper sulphate,	10	Three spores germinated.
18	Copper nitrate,	10	Five spores germinated.
18	Potassium chromate,	10	Failure.
48	Atropine,	100	Failure.
48	Aloin,	100	Failure.
48	Aloin,	10	Not very good.
48	Cocaine,	100	Failure.
29	Broth of wheat leaves,		Success—long germ tubes.
29	Neutralized urine,		Only a few spores germinate—long germ tubes.
29	Non-neutralized urine,		Very few spores germinated.
29	Milk,		Success.
44	Wheat leaf broth,		Success.
44	Beef broth,		Success.

The next table shows results obtained with uredospores of *Puccinia graminis* Pers.

TIME (hrs.)	MEDIUM.	STRENGTH.	RESULTS.
7	Copper sulphate,	10	Poor, but not an entire failure.
7	Hydrogen peroxide (0.3 per cent. sol.),	10	Good.
7	Potassium sulphide,	10	Good.
42	Hydrogen peroxide (0.3 per cent. sol.),	10	Failure.
48	Aloin,	10	Success.
48	Chloral hydrate,	10	Failure.
48	Atropine,	10	Failure.
67	Potassium bi-chromate,	1	Not many spores germinated.
67	Salt,	10	Success.
67	Cinnamon decoction,	71.4	Very few spores germinated.
48	Camphor (commercial solution),	100	Success.
48	Tannic acid,	10	Failure: swells the germ pores.
48	Salicylic acid,	100	Failure: decolorizes the spores.
48	Morphine,	100	Failure.
48	Morphine,	10	Failure.
48	Chloroform,	10	Failure.

In the following table, including the most extensive experiments of all, are shown the results obtained with uredospores of *Puccinia coronata* Corda.



TIME. (hrs.)	MEDIUM.	STRENGTH.	RESULTS.
22	Potassium chromate.	10	Partial failure; a few spores germinated.
22	Copper sulphate.	10	A few spores germinated.
24	Copper nitrate.	10	Very few spores germinated.
22	Copper acetate.	100	Total failure; decolorizes, then discolorizes the spores.
22	Lead acetate.	10	Total failure.
22	Ferric chloride.	10	Failure; colors spores green.
22	Potassium permanganate.	10	Success.
23	Corrosive sublimate.	1	Failure.
23	Potassium bi-chromate.	10	Failure; contracts and shrivels spores.
24	Hydrogen peroxide (0.3 per cent. sol.).	10	Success; very free germination.
24	Potassium sulphide.	10	Success; better than in water.
27	Ammonium sulpho-cyanide.	10	Success; very free germination.
27	Chrome alum.	10	Success; very free germination.
27	Potassium chromate.	10	Three spores germinated.
24	Copper chloride	10	Failure; decolorizes spores.
24	Potassium sulphide.	10	Success.
24	Potassium sulphide.	100	Failure.
24	Lead acetate.	10	Not many spores germinated; germ tubes stunted.
24	Copper acetate.	10	Failure.
47	Sodium arsenite.	10	Success.
47	Potassium chromate.	10	A few spores germinated.
47	Copper nitrate.	10	A few spores germinated.
48	Ammonium carbonate.	10	Success; very free germination.
48	Ferric chloride.	10	Very few spores germinated.
52	Sodium thio-sulphate.	10	Success.
52	Potassium per-manganate.	10	Success; not very free germination, however.
16	Lead acetate.	10	Very few spores germinated.
16	Ammonium carbonate.	100	Success; but germination not very free.
16	Magnesium chloride.	10	Success.
16	Ammonium sulpho-cyanide	10	Success.
16	Chrome alum.	10	Fairly well.
16	Sodium thio-sulphate.	100	Success.
27	Magnesium chloride.	10	A few spores germinated.
25	Potassium chromate.	10	Failure.
25	Sodium thio-sulphate.	100	Success; but germination not very free.
25	Nitric acid.	68	Failure.
25	Nitric acid.	6.8	Success; very free germination.
25	Corrosive sublimate.	1	Failure; decolorizes spores.
25	Potassium sulphide.	10	Success.
25	Acetic acid.	10.8	Failure.
25	Acetic acid.	0.54	Success.
18	Sulphuric acid.	10	Failure; decolorizes spores.
18	Potassium cyanide.	1	Success; very free germination.
52	Potassium cyanide.	10	Failure.



TIME. (hrs.)	MEDIUM.	STRENGTH.	RESULTS.
19	Potassium bi-chromate.	1	Fairly well.
24	Potassium sulphocyanide.	10	Five spores germinated.
26	Copper acetate.	1	A few spores germinated.
26	Copper sulphate.	1	Very few spores germinated.
26	Corrosive sublimate.	0.1	A few spores germinated.
26	Potassium bi-chromate.	1	Success, but not very free: swells out germ pores.
26	Copper nitrate.	1	Failure.
26	Lead acetate.	1	Six spores germinated.
21	Copper sulphate.	1	Success.
21	Potassium chromate.	10	Success.

Germinations of other species were not sufficiently extensive to justify tabulation. *Æcidium Fraxini* Schw. failed to germinate in copper sulphate (1:1,000) in twenty-six hours, and in potassium bi-chromate (1:1,000) same time, but germinated successfully in the latter solution with a strength of 1:100,000, producing peculiar short bulb-like germ tubes, for illustration of which, see pl. XXXVII, fig. 6. Quite a number of summer spore-forms, including various aecidia and uredines, germinated more or less freely in weak solutions (five to ten per cent.) of sugar and honey. In only one instance have I been able to grow the spermatia. The spermatia of *Uredo Cæoma-nitens* Schwein. budded sparingly, on May 31, 1893, after twenty-four hours in a dilute solution of honey, but would not germinate in water. This is in confirmation of the results obtained by Cornu<sup>2</sup> and Plowright.<sup>3</sup>

A careful comparison of the results above given, justifies certain conclusions of interest and importance. (1) Compounds containing mercury, copper, iron, lead and chromium (where these elements are in sufficient proportions), and strong acids, are *inimical* to the growth of Uredineæ. (2) Compounds containing oxygen, sodium, potassium, magnesium, sulphur and probably carbon and ammonium, in great proportions, are *favorable* to the growth of Uredineæ. (3) Alkaloids are injurious to the growth of Uredineæ. The compound radical cyanogen, might properly come under group (1), for, although the sulpho-cyanides of ammonium and potassium are not unfavorable, such fact may be explained by

<sup>2</sup>Bull. de la Soc. de Bot. de France xxiii (1876). 120-121.

<sup>3</sup>British Uredineæ and Ustilagineæ, 14-16.



the presence of the favorable element sulphur, not present in potassium cyanide. Again, while bi-chromate of potassium is extremely injurious, chrome alum is rather favorable, which may be explained by the fact of there being such a small proportion of chromium in the latter compound, compared with its amount in the former. The effect of the element nitrogen is hardly determined by these experiments, but the majority of the facts seem to point to its being injurious, since its presence in alkaloids makes the only difference, as far as mere composition is concerned, between these compounds, which are injurious, and the carbohydrates which are favorable; and since it is the greater constituent, by weight, in the unfavorable radical, cyanogen. The correctness of this idea would tend to overthrow the opinion, often expressed, that an excess of nitrogenous compounds in soils is favorable to the growth of rust. Another conclusion to be derived from the results of these experiments, is (4) that potassium sulphide and sodium hyposulphite, common fungicides, are likely to be entirely useless for the prevention of rusts, since the spores grow readily in solutions of these compounds, even with the latter in a solution of 1:100.

Dr. E. Wüthrich,<sup>4</sup> the only one, as far as I know, who has experimented in this same line, has had results very similar to my own, in so far as he has reported them. In his experiments with uredospores of *Puccinia graminis* Pers., germination took place in a solution of potassium nitrate of fifty per cent. strength. I did not use this salt at all, but it will be noted that it contains the favorable element, potassium, that I have already mentioned. On the other hand, he has not reported the employment of potassium bi-chromate, one of the salts having the most injurious effect upon germination in my own experiments. But where we have used the same compounds, the results have been practically the same.

## 2. Vitality and vigor of the summer spore-forms.

Uredospores and æcidiospores have much greater powers of endurance than have usually been ascribed to them. Wüthrich, in his article above cited, makes the following statement with respect to the resistance of uredospores of *Puccinia gra-*

<sup>4</sup>Ueber die Einwirkung von Metallsalzen und Säuren auf die Keimfähigkeit der Sporen einiger der verbreitetsten parasitischen Pilze unserer Kulturpflanzen. Zeits. f. Pflanzenkrankheiten II (1892). 84-86.



*minis* to the action of various solutions in their germination, compared with spores of other fungi, with which he also experimented: "The spores of various fungi show unlike powers of resistance against solutions of metal-salts and acids. The conidia of *Peronospora viticola* prove to be the most susceptible of the forms investigated. Then, following these, in the order of decreasing sensibility, are the conidia of *Phytophthora infestans*, æcidiospores of *Puccinia graminis*, conidia of *Claviceps purpurea*, spores of *Ustilago Carbo*, and uredospores of *Puccinia graminis*."<sup>5</sup> According to this statement, uredospores have, comparatively, great powers of resistance to various solutions, and there is the further fact that æcidiospores are much less resistant than uredospores, both of which facts are further established by my experiments, so far as I have gone.

They are also similarly resistant to extremes of cold. It was already well known that the mycelium of *Puccinia Rubigo-vera* can pass the winter without injury, in the tissues of its host.<sup>6</sup> Experiments at this station have further proved that even the *uredospores* are preserved during the winter months, and may be taken from the field in *any month* of the year and easily germinated.<sup>7</sup>

A plant of *Carex (vulpinoidea?)* bearing both uredospores and teleutospores of *Puccinia Caricis* (Schum.) Rebent. was transplanted to the green-house from out doors in December, 1892, and uredospores, taken from this plant in January following, readily germinated, producing germ tubes of rather peculiar form—wide, and often branching (pl. XXXVIII, fig. 11). Germination would probably have taken place at the time of transplanting, but was not attempted. *Æcidium tuberculatum* Ell. & Kell. is still producing æcidiospores on *Callirhoe involucrata*, out doors, here at Manhattan, at the time of this writing (October 15th), and Mr. E. Bartholomew, of Rooks co., Kan., tells me that he has seen in December æcidiospores on specimens of this host growing close by a large snowdrift. In the spring, æcidiospores of this species begin forming about the first day of April. Æcidiospores of *Æcidium Pentstemonis* Schwein., and uredospores of *Uromy-*

<sup>5</sup>l. c. translated from p. 93.

<sup>6</sup>See Sorauer, Handbuch der Pflanzenkrankheiten, ed. 2. II. 216; and Bolley, Bull. Agric. Exp. Sta. Ind. 26, 13 and 19.

<sup>7</sup>See Bull. Kan. Agr. Exp. Sta. 38, 11, for full discussion of this matter.



*ces Zygaeni* Pk. and *Puccinia Hieracei* (Schum.) Mart. begin forming also about April 1st.

It is not always necessary that the summer spore-forms should be fresh in order to germinate readily, although fresh spores usually germinate best. Spores of *Æcidium pustulatum* Curt. and *Uredo Cæoma-nitens* Schwein. collected at Lawrence, Kan., May 19, 1892, and placed in drop cultures at this station the next day, germinated freely in 31 hours, producing vigorous germ tubes. I have often germinated uredospores, after they had lain in the collecting can one or even two days. Prof. B. T. Galloway,<sup>8</sup> several years ago called attention to the great scarcity of ash rust, *Æcidium Fraxini* Schwein., in 1888, and to the fact that various attempts, by the Division of Vegetable Pathology, to germinate it in different media, were only partially successful at that time. At the same time he suggested the question whether the rust might not germinate more readily in seasons of great abundance. As bearing upon this question, it may be of interest to say that in the summer of 1892 the rust was extremely abundant here, particularly on young trees, attacking the twigs as well as the leaves and sometimes entirely destroying large portions of the branches for a distance of two feet or more. At the same time, the spores were easily and repeatedly germinated in ordinary watch glass cultures, although it had been difficult for me to germinate them at other times. I think I have observed the same fact with respect to other species.

As to the length and rapidity of growth of germ tubes, there is great variation among the different species. Those of the uredospores of *Puccinia Rubigo-vera* represent about the average length, so far as my observations have extended. Those of *Puccinia Hieracei* are a little longer than the average. (See pl. XXXVIII, fig. 14.) Those of *Puccinia graminis*, and of various rusts on grasses, are much longer. The one figured in pl. XXXVIII, fig. 12, has a length of 1.075<sup>mm</sup>, and is but little, if any, longer than the average for that particular culture, of seventy-two hours. Uredospores of *Puccinia Sorghi* Schwein. sometimes show no indication of germination during the first day, but finally, at the end of forty-eight hours, produce germ tubes of very fair length. Other species seem to produce their entire growth in twenty-four

<sup>8</sup>Journ. Mycol. v (1889). 95.



hours. Germination in the same species will vary greatly, according to the conditions. Fig. 8, pl. XXXVII, shows the progress that various spores have made at the end of four hours. On January 25, 1892, uredospores of *Puccinia Rubigovera*, taken from outdoor plants, germinated in *two hours* in warm water, producing germ tubes  $50\mu$  long. In this case germination could actually be seen going on. The germ pores began swelling almost immediately on being placed in the water.

### 3. A new method of producing sporidiola.

In the course of my experiments with the germination of teleutospores, I have observed a new process in the formation of sporidiola<sup>9</sup> not hitherto mentioned so far as I know.<sup>10</sup>

In the ordinary process it is well known that the sporidiola are stalked, or attached to pedicels of peculiar shapes—usually very much narrowed at the point of attachment. These are well illustrated in pl. XXXVIII, fig. 15 and pl. XXXIX, fig. 16. The pedicels may arise from various points for some distance along the promycelium, or they may be almost in clusters, originating from points rather close together. The process that I shall describe is very different, and is as follows: At first the promycelium presents the ordinary appearance, but presently there is an evident disposition in the terminal portion to separate into well-marked divisions. The protoplasm concentrates in these divisions; constrictions form between them; the divisions, at first much longer in the direction of the promycelium, become shorter and wider, then rounded, and finally, separate from the remaining portion of the promycelium, as free spherical bodies, by the ordinary process of acrogenous abjunction. A half dozen or more may be produced in succession from the same promycelium. Of course those nearest the end are the oldest, and may be in the process of separation while those farthest back are just beginning to form.

We have in this process a production of *catenulate* sporidiola, in contradistinction to the *pedicelled* sporidiola hitherto observed. I have observed this process in three species, *Puccinia Grindeliæ* Pk., *P. variolans* Hark. (so-called) on *Aplo-*

<sup>9</sup>I use this term, in preference to "sporidia," in order to avoid confusion with the sporidia of ascomycetes and other groups. It is suggested by Saccardo, in "Rathschläge für die Phytographen insbesondere die Kryptogamisten," Hedwigia xxx. (1891). 56-59, and used by De Toni in Sacc. Syll. vii. 528.

<sup>10</sup>Lagerheim possibly refers to it (Jour. Mycol. vii. 46-47), but he observed only LEPTO-UREDINEÆ, in which I do not find this process.



*pappus spinulosus*, and in the mesospores of *P. Sporoboli* Arth. This process of germination may prove to be of importance in classification, and help to clear up the existing confusion regarding some of our western so-called LEPTO-PUC-CINIÆ on composites. I am not yet prepared, nor have I the space in this article, to make any statements, on this basis, concerning the systematic position of such species.

In two other cases, those of *Puccinia Malvastri* Pk. and the *Puccinia* of *Lygodesmia juncea*, the indications were that catenulate sporidiola would be formed, but germination ceased before reaching that stage. The germination of *Puccinia Sporoboli* was the most remarkable. In this case, not a single two-celled spore germinated while the mesospores germinated profusely, uniformly producing catenulate sporidiola.

All these species, except *Puccinia Sporoboli*, were collected in October, 1892, and germinated after being kept in paper pockets till April, 1893. It may be said that this fact may have had something to do with the peculiar germination of these species, and possibly it did, but *Puccinia Phragmitis* (Schum.) Körn. and *Puccinia Redfieldiæ* Tracy were collected at the same time, and after passing through the same treatment, germinated in April, also, but with the production of the ordinary pedicelled sporidiola; besides, *Puccinia Sporoboli* was taken from fresh out-door material.

I have constantly had much aid from Prof. A. S. Hitchcock, in the course of my investigations, particularly in the selection and preparation of the various solutions used in germination.

*Kansas Experiment Station, Manhattan.*

#### EXPLANATION OF PLATES XXXVII—XXXIX.

Fig. 1. Germination of uredospores of *Puccinia coronata* Corda, in ammonium carbonate (1:1000), after 48 hrs.  $\times$  247.

Fig. 2. Condition of the same, in potassium bi-chromate (1:1000), after 23 hrs.  $\times$  333. Spores much contracted and shrivelled.

Fig. 3. Germination of uredospores of *Puccinia Rubigo-vera* (DC.) Wint. in sodium chloride (1:1000), after 21 hrs.  $\times$  247.

Fig. 4. Germination of uredospores of *Puccinia coronata*, in sodium thiosulphate (1:1000), after 52 hrs.  $\times$  333. Germination vigorous.

Fig. 5. Germination of *Æcidium Fraxini* Schwein., in watch-glass water culture, after 3 days, June 15, 1892.  $\times$  333.

Fig. 6. Germination of the same, in potassium bi-chromate (1:100,000), after 28 hrs.  $\times$  333.

Fig. 7. Condition of uredospores of *Puccinia coronata* in lead acetate (1:1000), after 22 hrs.  $\times$  333.

Fig. 8. Germination of uredospores of *Puccinia Rubigo-vera*, in water, after 4 hrs.  $\times$  200. Shows the various stages in germination.



- Fig. 9. Germination of the same, in water, after 44 hrs. Jan. 21, 1893.  $\times 200$ . Spores taken from volunteer wheat, out doors.
- Fig. 10. Spores taken from dead leaves of volunteer wheat, out doors, Feb. 25, 1893. Seem to have germinated on the wheat, and further growth checked by the cold.  $\times 247$ .
- Fig. 11. Germination of uredospores of *Puccinia Caricis* (Schum.). Rebent. in neutralized urine, after 50 hrs. Jan. 1893,  $\times 333$ . Spores from green-house plant, transplanted from out doors Dec. 1892.
- Fig. 12. Germination of uredospores of *Puccinia graminis* Pers. in water, after 72 hrs. Germ tube measures 1.075mm.  $\times 247$ .
- Fig. 13. Germination of *Uredo Cæoma-nitens* Schwein. in water, after 31 hrs. Put in drop culture next day after collection, May 19, 1892.  $\times 333$ .
- Fig. 14. Germination of uredospores of *Puccinia Hieracei* (Schum.) Mart. in water, after 24 hrs. Apr. 30, 1892.  $\times 167$ .
- Fig. 15. Germination of teleutospores of *Puccinia Redfieldiæ* Tracy, in water, after 46 hrs April 29, 1893, collected Oct. 1892.  $\times 247$ . One mesospore is shown to be germinating.
- Fig. 16. Germination of teleutospores of *Puccinia Phragmitis* (Schum.) Körn. in water, after 4 days, April 1893, collected Oct. 1892.  $\times 247$ .
- Fig. 17. Germination of teleutospores of *Uromyces Polygoni* (Pers.) Fuckel. in water, after 46 hrs. April 29, 1893, collected Oct. 1892.  $\times 247$ .
- Fig. 18. Germination of teleutospores of *Uromyces Sporoboli* Ell. & Ev. in water, after 48 hrs. Apr. 17, 1893.  $\times 333$ .
- Fig. 19. Germination of teleutospores of *Puccinia Sporoboli* Arth. in water, after 48 hrs. Apr. 17, 1893. *a*, sporidiola.  $\times 247$ . Only the mesospores germinated, producing catenulate sporidiola.
- Fig. 20. Germination of teleutospores of *Puccinia Grindeliæ* Pk. in water, after 20 hrs. Apr. 24, 1893, collected Oct. 1892. Producing catenulate sporidiola. *a*, sporidiola.  $\times 247$ .
- Fig. 21. Germination of teleutospores of *Puccinia variolans* Hark. of *Aplopappus spinulosus*, in water, after 19 hrs., producing catenulate sporidiola. *a*, terminal portions of promycelia, isolated, showing sporidiola in the process of abstricting. *aa*, sporidiola free.  $\times 247$ .

## Botanical notes from Bainbridge, Georgia. I.

AUGUST F. FOERSTE.

WITH PLATE XL.

### Rootstocks penetrating the ground vertically.

Every one knows how roots penetrate the ground. The minute terminal rootlets find their way along crevices between the particles forming the earth, and the increasing bulk of the root pushes these particles aside. It is different with a rootstock. Its existence on germination begins near the surface of the ground and if it penetrates the ground vertically it must penetrate it backwards.

Where plants grow in marshy soil, as in the case of *Symplocarpus foetidus*, this is readily understood, but when the ground is more compact it is quite another matter. It must